Influence of the cellobiase enzyme activity by microwave radiation

Erika Lakatos – Attila J. Kovács – Viktória Kapcsándi – Miklós Neményi
University of West Hungary, Faculty of Agricultural and Food Sciences
Hungary, Mosonmagyaróvár, Vár 2.
kapocs@mtk.nyme.hu

ABSTRACT

Our research aim was to enhance the activity of β-glucosidase enzyme in connection to the 2nd generation bio-ethanol production by using only physical methods. In order to study the non-thermal effect of microwave treatment samples were heated with the same parameters as microwave heating by conventional conductive method (hot plate) as standard. The enzyme activity changes were followed by the increased glucose concentration. Based on the results the produced glucose of the microwave treated solution was 26% higher than in the solution heated up on a hot-plate. After treatment in each case solution were supplemented into enzyme-substrate-buffer system. The treating only the buffer solution after treatment with the enzyme activity was an average 16% compared to the control sample. If the substrate (cellobiose) and buffer were treated together and after this treatment the enzyme was added to the solution. In this case there was not difference compared with the aforementioned measurements. In the next test series, the enzyme-buffer suspension was heated in a microwave and hot plate and then was added to the substrate. In this case, in the solution that was heated by microwave the enzyme activity was an average 18% higher that the hot plate heated solution. Hence, it can be concluded that microwave affected not only the buffer solution but the enzyme, too. Supplementary measurements were carried out in which the change of enzyme activity was investigated directly after treatment, and 48 and 96 hours later. Based on our results the microwave-treated enzyme can broken the cellobiose 20% more effectively 48 hours and 96 hours after treatment than in the hot-treated enzyme.

Key words: microwave, enzyme, cellobiose, bioethanol

1. INTRODUCTION

In the Earth approximately $4xl0^{10}$ tons cellulose produced each year, the total volume of $7x10^{11}$ tons, so the most abundant carbohydrates from plant biomass source. Secondary sources of biomass, such as agricultural and industrial wastes also contain huge amounts of cellulose (Coughlan and Mayer, 1992). In recent years increasingly has grown the interest of industrial use of cellulose. This will be the raw material of constantly renewable energy source to the chemical industry, food industry and not it least can be the basis for the preparation of bio-ethanol (Laszlo et al., 2007). The latter are particularly important area for the second generation, hemicellulose – lignocellulose, too according to some authors (Chen and Qiu, 2010; Balat 2011) - based targeting efficiency of bioethanol processing research. As a first step in the production of ethanol cellulose has converted to glucose. A break down happen at high temperature and high acid pressure or by using enzymes (Réczey et al., 1996). The latter method has increasing role on the basis of environmental and energy concerns. During the process the cellulose enzyme system is applied, which avoid the side-product formation and higher glucose yields are achieved than conventional acid hydrolysis process. The successful of the cellulosic ethanol production depends on largely of the lignocellulose pretreatment (Zhu et al 2006; Xu et al 2011..; Lu et al 2011) and the efficient cellulase enzyme complex applications, too.

In the cellulose enzyme system (endoglucanase, cellobiohydrolase, cellobiase) the role of the β-glucosidase (cellobiase) is the breakdown of the intermediate cellobiose, which is the inhibitor of the rest of the enzyme system. In this way the role of β-glucosidase non-negligible in the enzymatic breakdown of cellulose (Gasztonyi and Lásztity 1992; Jager, 2003).

According to references to the change of enzyme activity used successfully low-power microwave radiation (Szabo et al. 1998; Parker et al. 1996; Lin and Lin 1998;. Bradoo et al, 2002; Nogueira et al. 2010.) Based on these results we tried to answer the question to the low-power microwave radiation impact the function of the β-glucosidase / cellobiase enzyme. Our aim was to elaborate a treatment protocol, which we wanted to achieve higher glucose by increasing the activity through enzyme used. During the hydrolysis released from glucose entering into the alcoholic fermentation processes can be source of nutrients for the yeast. The hydrolysis and fermentation individually and in case of sufficient glucose together (simultaneously) can be realized.

Despite the fact that the simultaneous process due to the participant enzymes and micro-organisms involved requires a more complex system, time and cost-effective ethanol production can be achieved (Zhu et al 2005; Nikolic et al 2009).

2. MATERIALS AND METHODS

During the experiments sodium-acetate acetic-acid buffer solution (0.1 M, pH 4.6) was used. In a solution suspended 4 g of D - (+) - cellobiose substrate and 2 ml of 1,4- (1,3: 1,4) – B-D-Glucan-4glucano-hydrolase (Sigma-Aldrich, ATCC 26921) enzymes. The measurements were performed six replicates

The treatments performed with Panasonic NNF 653WF microwave oven (Quebec, Canada) completed with FISO fiber optic. During the microwave irradiation of enzyme suspension we want to realize even homogeneous distribution, so we tried to adjust the uniformly prevail of microwaves effect, therefore the treatment was carried out using water traps. The center of the rotating plate paced 60 mm high and 85 mm diameter Teflon sample vessel whatever it takes 200 ml enzyme suspension. Around the sample vessels we spent 90 g of tap water in four pieces 10 mm high, 38 mm diameter vessels, which therefore formed a water trap. A significant part of the irradiated energy (submitted magnetron power 50W) absorbed (≈ 83%) of the water trapped in (Lakatos et al., 2005), so in spite of the 25-minute exposure time increased the test samples only to 45 °C temperature (heating rate v = 1.8 ° C / min, the material dissipated 42.5 mW / mL). In order to we can compare it microwave and through conventional hot plate heating effect (that is to microwaves does not consider the thermal effect), as a control the same enzyme-substrate suspension is heated type Yellowline Mst basic C hot plate magnetic stirrer, similar heating parameters (initial temperature, time of heat treatment, heating rate) used.

The change of enzyme activity was monitored by the change of glucose concentration in the solutions. In preliminary experiments was used standard glucose solutions and glucose GOD / PAP stable liquid (Diagnosticum Ltd.) We set up the linear relationship between the glucose concentration in the standard solutions and the measured absorbance of solutions in 505 nm ($R^2 = 0.998$) in a spectrophotometer (Hitachi UV / VIS photometers).

$$Cg = 3.6099ABS – 0.2232 \quad (1)$$

Cg: emerging amount of glucose (g/L)

ABS: absorbance value of the solution at 505 nm

During the experiments immediately after the treatments were taken from 1 ml sample from the microwave and hot-plate heated samples, to which added 10 ml of glucose GOD / PAP reagent. The resulting solution was incubated at 37 °C in water bath for 10 minutes and then the absorbance of the solutions at 505 nm was measured. The obtained absorbance value substituting to the calibration line (1) we determine the equation of glucose content of solutions.

The microwave and hot plate heat-treated samples were stored at 37 ° C water bath. During the storage and after 30, 60, 120 and 180 min were taken from 1 ml sample and as described above were determined the glucose content of the samples. During the measurements the statistical analysis was carried out with 95% significance level.

3. RESULTS AND DISCUSSION

After microwave and hot plate treatment were sampling for enzyme suspension immediately (0 min) and after the incubation at 37 ° C for 30, 60, 120 and 180 minutes and the glucose concentration was calculated using the absorbance values of the solutions. Fig. 1 shows the average results. The microwave effect was approximately 26% more intense on the enzyme activity than the same conditions, but in the case of a hotplate heated samples.

In the following experiments we measured the difference between the two heating modes (microwave, hot plate), when we treated with only the buffer solution, and after the heating added the substrate and the enzyme. The measurements were carried out in this case six replicates, too (Figure 2). It is visible that if only the buffer solution was heated and then added the enzyme and the substrate, there is also difference in glucose content between the microwaves and the hot plate heated samples. In case of the microwave irradiated sample which was added enzyme produced average 16% more glucose than the hotplate heated sample. It should be noted that this series of measurements after 180 minutes of treatment had no measurable difference between the samples in respect of glucose content. Based on these results it can be stated that the non-thermal effect of microwave prevail on the buffer, too. (In order to clarify the impact of electromagnetic radiation on buffer more measurements are necessary.) We investigated the effect of combined heat-treatment of buffer and the substrate as well. These results showed similar values as only after treatment the buffer measurements (mean difference even at 16%). The reason lies in that the microwave radiation can not to brake down the substrate chemical bonds used on our treatment microwave power (Datta Anantheswaran 2001), in other words microwave cant batter down cellobiose, so there was no

difference between the two series of measurements. The following were examined the changing of together heat treated buffer and enzyme samples. The heat treatments were made as above to previously describe. In this case after the heat treatment was added the substrate to the solutions and measuring the glucose concentration. During this measurements we could detected difference between the various ways heated samples. We measured average 18% more glucose content in case of microwave treated samples. The measurements were carried out in this case six replicates, but in this case we can't detected difference after 30 minutes of treatments, however the enzyme activity was measurable after 180 minutes the treatment (Figure 3).

In the last series of measurements we compared the microwave treated and hot plate heated, that after treatment at 48 and 96 hour in 8 °C incubated enzyme suspension activity with recently treated samples. Fig. 4 illustrated the performed average six repetitions results.

In this case we taken samples after 60, 120, and 180 minutes from the solutions (after 30 minutes the insertion of the substrate, there was no measurable difference in the glucose content of the solutions concerned). The statistical evaluation of the measurement results the t-test was applied. We could demonstrate significant differences between the microwave and the hot plate heated samples (p=5%). Based on these results it can be stated that the microwave heated cellobiase enzyme activity increased after 96 hours in compared to the hotplate treated cellobiase enzyme suspension. The rate of increasing both after 48 and 96 hours approximately 20%.

Based on our results the low-power electromagnetic radiation alter the enzyme activity. In our case during the studies we have achieved higher glucose concentration the microwave-treated samples than the conductive, hot plate treatments. The formed glucose is potential nutrient source for in the fermentation process participant yeast, this is enable a higher concentration for faster and / or more efficient ethanol production.

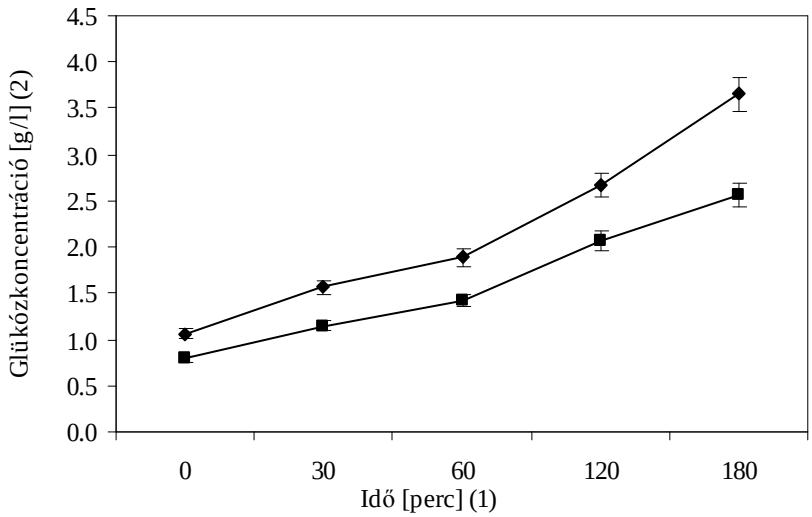
The buffer and enzyme treatment without substrate it is important, that if it is possible to increase the activity of enzymes with microwave irradiation, can be prepared enzyme formulations which can be stored even after the irradiation, and the using is later possible, too.

REFERENCES

1. Balat M. (2011). 'Production of bioethanol from lignocellulosic materials via the biochemical' **Energy Conversion and Management**. 52, 858-875.
2. Bradoo S., Rathi P., Saxena R.K., Gupta R. (2002). 'Microwave-assisted rapid characterization of lipase selectivities' **Journal of Biochemical,** Biophysical Methods 51, 115-120.
3. Chen H., Qiu W. (2010) 'Key technologies for bioethanol production from lignocellulose' Biotechnology Advances. 28, 556-562.
4. Couglan M.P., Mayer F. (1992). 'In the Prokaryotes: handbook on the biology of bacteria' **Chapman and Hall, New York.**
5. Datta A.K., Anantheswaran R.C. (2001) 'Handbook of Microwave Technology for Food Applications' Marcel Dekker, Inc., New York.
6. Gasztonyi K., Lásztity R. (1992). 'Élelmiszer-kémia I.' **Mezőgazda Kiadó, Budapest.**
7. Jáger Sz. (2003). 'Aspergillus carbonarius-ból izolált extracelluláris β-glükozidáz működési mechanizmusának vizsgálata' **PhD dissertation, Debreceni egyetem, Debrecen**.
8. Lakatos E., Kovács A.J., Neményi M. (2005). 'Homogenious microwave field creation' Hungarian Agricultural Engineering. 18, 80-81.
9. László Zs., Beszédes S., Kertész Sz., Hodúr C., Szabó G., Kiricsi I. (2007). 'Bioethanol from sweet sorghum' Hungarian Agricultural Engineering 20, 15-17.
10. Lin G., Lin W.Y. (1998). 'Microwave promoted lipase catalyzed reactions' Tetrahedron Letters 39, 4333-4336.
11. Lu X., Xi B., Zhang Y., Angelidaki I. (2011). 'Microwave pretreatment of rape straw for bioethanol production: Focus on energy efficiency' Bioresource Technology. 102, 7937-7940.
12. Nikolic S., Mojovic L., Rakin M., Pejin D. (2009). 'Bioethanol production from corn meal by simultaneous enzymatic saccharification and fermentation with immobilized cells of *Saccharomyces cerevisiae* var. *ellipsoideus*' Fuel 88, 1602–1607.
13. Nogueira B.M., Carretoni C., Cruz R., Freitas S., Melo A.P., Costa-Félix R., Pinto J.C., Nele M. (2010). 'Microwave activation of enzymatic catalysts for biodiesel production' Journal of Molecular Catalysis B: Enzymatic 67, (1-2) 117-121.

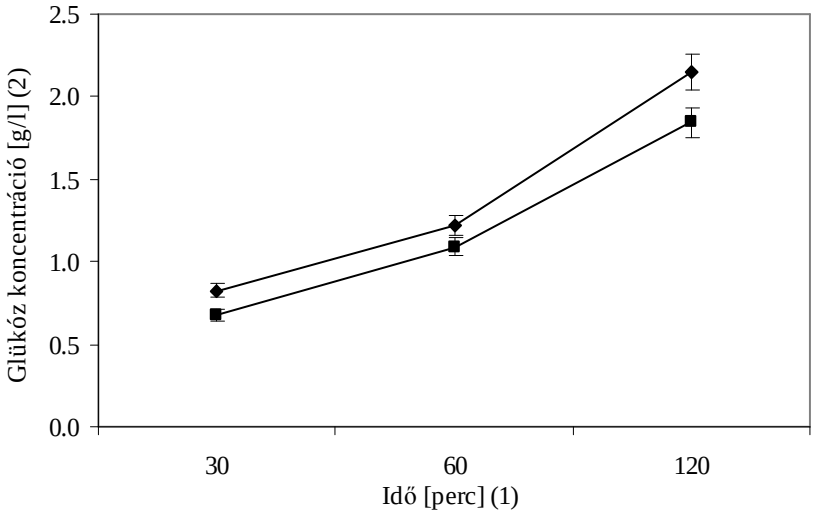
14. Parker M.C., Besson T., Sylvain L., Legoy M.D. (1996). ‘Microwave radiation can increase the rate of enzyme-catalyzed reactions in organic media’ Tetrahedron Letters 37, (46) 8383-8386.
15. Reczey K., Szengyel Zs., Eklund R., Zacchi G. (1996). ‘Cellulase production by *T. reesei*’ Bioresource Technology 57, 25-30.
16. Szabó G., Rajkó R., Kovács E., Vidal C. (1998). ‘Designed experiments for reducing antinutritive agents in soybean by microwave energy’ Journal of Agricultural and Food Chemistry 45, 3565-3569.
17. Xu J., Chen H., Kádár Zs., Thomsen A.B., Schmidt J.E., Peng H. (2011). ‘Optimization of microwave pretreatment on wheat straw for ethanol production’ Biomass and Bioenergy 35, 3859-3864.
18. Zhu S., Wu Y., Yu Z., Zhang X., Wang C., Yu F., Jin S. (2006). ‘Production of ethanol from microwave-assisted alkali pretreated wheat straw’ Process Biochemistry 41, (4) 869-873
19. Zhu S., Wu Y., Yu Z., Zhang X., Wang C., Yu F., Jin S., Zhao Y., Tu S., Xue Y. (2005). ‘Simultaneous Saccharification and Fermentation of Microwave/Alkali Pre-treated Rice Straw to Ethanol’ Biosystems Engineering 92, 2229-2235.

Figure 1 The activity changes of cellulase enzyme, substrate (D – (+) - cellobiose) in buffer suspension treated by microwave (◆) and conventional heat treatment (■)



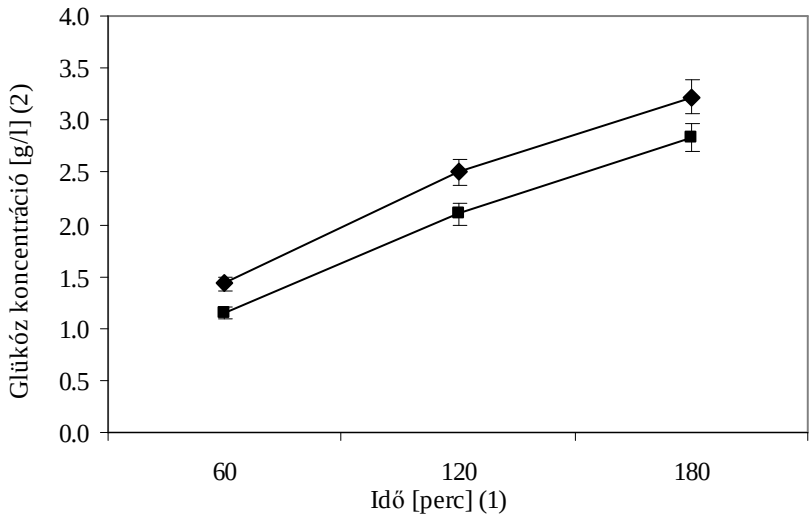
(1) Time [min]
(2) Concentration of glucose [g/l]

Figure 2 The change of glucose content only buffer soluton after microwave (◆) and conventional heat treatment (■)



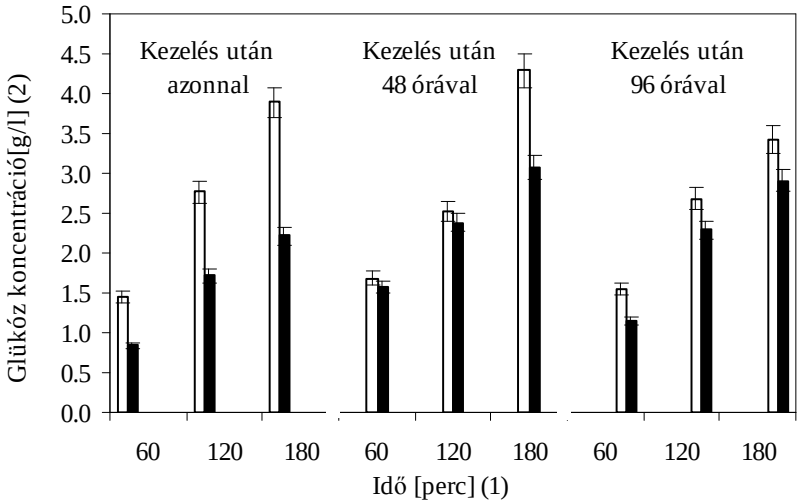
(1) Time [min]
(2) Concentration of glucose [g/l]

Figure 3 The change of glucose content only enzyme-buffer soluton after microwave (◆) and conventional heat treatment (■)



(1) Time [min]
(2) Concentration of glucose [g/l]

Figure 4 The change of enzymeactivity in microwave (□) and conventional heat treated (■) enzyme suspension direct after treatment, 48, and 96 hours later



(1) Time [min]
(2) Concentration of glucose [g/l]
(3) Directly after treatment
(4) 48 hours after treatment
(5) 96 hours after treatment